

27820 Course Schedule in short

In general: We will be running a total of 5 biofilm/flow cell experiments. Each team will be responsible for one flow cell system. This means that the team must assemble the system, sterilize it, and fill it with medium according to the instructions. Furthermore, the team must daily check that the medium supply is sufficient and empty the waste. All teams will inoculate and work with flow channels from all of the six systems, so the "responsibility" is limited to the above mentioned maintenance tasks. Teams that are going to inoculate strains in the free exercise biofilm system will also be responsible for media supply and waste in this system.

The free exercise is what the name implies: free. This means you can decide which experiments you want to perform and which analyses you want to do. The work on this exercise is administered almost entirely by yourself (there are, however, some time slots assigned to this). *Bring your own samples of interest for the free exercise or sample from the local area during the first part of the course.*

Friday 19/8:

- 8:30 Breakfast (optional).
- 9:00 The exercises and practical information
- 9:30 **Technical Talk: Janus Haagensen and Claus Sternberg: Biofilms and tools, confocal microscopy and COMSTAT, Imaris and FACS.**
- 10:30 Tour of the institute and space in the lab.
- 11:00 **Talk: Lars Jelsbak: Bacterial evolution in chronic infections.+ Intro to Clondiag exercise**
- 12:00 Lunch. (Lunch is on your own all days, except for 26/8 where pizzas are provided).
- 1:00 Building Biofilm setups (All in all we will work on 5 systems during the course, each team of 3 will be responsible for 1 system with respect to media preparation and waste removal, all teams will work on all systems)
Medium preparation for all biofilm systems.
- 3:00 The free exercise: Sampling of bacteria from your own chosen environment (contact lenses, soil, plants etc). Isolation of bacteria/plating and incubation at different temperatures.
- 4:00 Inoculation of already prepared and sterilized system 1 and 3.
- 5:00 End of day 1

Saturday 20/8:

- 9:00 **Technical Talk: Claus Sternberg: COMSTAT2**
- 9:30 Microtiter assay: inoculation of 2 plates, one that you will proceed with by hand and one that will be handled using a robot (*E. coli* and mutants, each team make their own plates).
The free exercise: Inspect plates and continue as you want from own ideas or guidelines in the manual material.
Confocal microscopy and image acquisition of the *E. coli* strains (biofilm system 1). Will take place most of the day one team at the time (images for COMSTAT).
- 12:00 Lunch. (bring your own lunch (cantina closed.)
- 1:00 Sterilization of biofilm system 2, 4, and 5.
- 1:30 **Talk: Anders Folkesson, Strain adaptation by repeated antibiotic treatment.**
- 2:30 MIC determinations of *P. aeruginosa* determined by different conc. of colistin in test tubes and using E-test (have to be used later in the course for biofilm experiments (system 3 and 4)),
- 5:00 Rounding up in the lab with things you did not manage during the day
- 6:00 End of day 2.

Sunday 21/8:

- 9:00 Images for COMSTAT using confocal microscopy, most of the day one team at the time (biofilm system 1).
Inoculation of biofilm system 2, 4 and 5 (if you have isolated strains you would like to see develop in a flow cell).
Start of diagnostic exercise (Multiplex PCR and plate prep)
Microtiter assay: Washing and reading (OD measurement and Crystal violet staining) as well as introduction to and starting up of the robot with your second microtiter plate.
- 12:00 Lunch. (bring your own lunch (cantina closed.))
- 1:00 **Technical Talk: Claus Sternberg: FISH**
- 1:30 Images for COMSTAT using confocal microscopy, most of the day one team at the time (biofilm system 1).
Diag. exercise contd.
Look at MIC determinations tubes and plates.
Free exercise (continued... QS, make plates for motility assay, biofilm assays, microscopy/morphology structures on plates ...).
- 6:00 End of day 3.

Monday 22/8:

- 9:00 **Talk: Claus Moser: Animal models in biofilm and CF research.**
- 10:00 COMSTAT on obtained images
Imaris treatment of images.
Diagnostic exercise: Antibiotic test. Team 1-3: Hybridization/detection
- 12:00 Lunch.
- 1:00 **Michael Kühn, Microenvironmental analysis of biofilms – techniques and concepts**
Addition of antibiotics to biofilm system 3.
Diag. exercise contd.
Analyzing data from COMSTAT.
Work on the free exercise (continued...).
- 5:00 End of day 4.

Tuesday 23/8:

- 9:00 Staining of biofilm system 3 with Propidium Iodid (*P. aeruginosa* +/- antibiotics and *P. aeruginosa* (Yfp)+ *P. aeruginosa* pilA (Cfp))
Diagnostic exercise: Insect plates. Team 1-3: Data analysis, Team 4-5: Hybridization/detection
Image acquisition of biofilms, system 3 continued during most of the day
Microtiter assay: PAO1 and mutants as in biofilm system 4.
Inspect biofilm system 2 before FISH plating and sorting tomorrow.
- 12:00 Lunch.
- 2:00 Image acquisition of biofilms, system 3 continued during most of the day
Diagnostic exercise contd.
Work on your free exercise (follow biofilm development if you have inoculated flow system 5 with your isolated strains)
Microtiter assay: PAO1 and mutants as in biofilm system 4.
Inspect biofilm system 2 before FISH, plating and sorting tomorrow.
Treat images using Imaris, COMSTAT,...

5:00 End of day 5.

Wednesday 24/8

9:00 Talk: Thomas Bjarnsholt, Quorum Sensing and QS inhibition.

10:00 Embedding and hybridization+harvesting and fixation and plating of biofilms (*P. aeruginosa* + *Acinetobacter*; biofilm system 2) (procedure continues throughout the whole day).
Addition of antibiotics to biofilm system 4.
Read micro titer assay on Elisa reader
Work on the free exercise (continued...)
Diagnostic exercise: team 4-5: Data analysis

12:00 Lunch

1.00 Talk: Klaus Kirketerp-Møller, Biofilms and wound infections.

2:00 Continue the work from biofilm system 2
Work on the free exercise (continued...).

6:00 End of day 6

Thursday 28/8:

9:00 Talk: Søren Molin, Biofilms, hypermutators and population diversity

10:00 Count plates from harvested cells from biofilm system 2
Image hybridized samples from biofilm system 2
FACS of hybridized single cells from biofilm system 2
Image acquisition of biofilm system 4

12:00 Lunch.

1:00 Image acquisition of biofilm system 4
Work on free exercise Data treatment, preparation of presentation.
Inspect the last samples. Treat images using Imaris.

6:00 End of day 7.

Friday 26/8:

9:00 Cleaning procedures for biofilm systems.

The last preparations for the presentations

12:00 Evaluation and presentation of results. Pizza and beers/soft drinks will be served.

4:00 End of course.

Biofilm Systems used.

Biofilm system 1: *E. coli* (traA), *E. coli* (F+), *E. coli* (traD) mutant, Image acquisition and COMSTAT (15 channel system, Media FB with Fe-EDTA+A-10. Addition of glucose+proline+thiamine).

Biofilm system 2: *P. aeruginosa* (CY5 blue probe) + *Acinetobacter* (Cy3 red probe): FISH, directly on biofilm and syto staining of harvested cells. Also plating of harvested cells on selective plates (PA will grow on 100 µg/ml Amp) (ACN will grow on 100µg/ml Strep) + FACS sorting/counting for estimation of ratio between the species after biofilm establishment harvesting and FISH. (15 channel system, Media FB with trace metals+ A-10. Addition of glucose).

Biofilm system 3: *P. aeruginosa* (Gfp) alone two channels +/- antibiotic treatment and *P. aeruginosa* (Yfp) + *P. aeruginosa* pilA (Cfp) for structure development, image acquisition after antibiotic treatment. (15 channel system, Media FB with trace metals+ A-10. Addition of glucose).

Biofilm system 4: POA1 mutants, antibiotic treatment and structure development. (15 channel system, Media FB with trace metals+ A-10. Addition of glucose).

Biofilm system 5: Biofilm system 5: Prepared for the free exercise (Participants own strains or strains isolated during the course). We will consider mediums dependent on the strains. Samples for AFM: *P. aeruginosa* (Flagella) and *E. coli* (Fimbria)

Time lapse will be shown as demonstration videos and explained during the CLSM sessions.